A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP5621.

Gestational Exposures to Phthalates and Folic Acid, and Autistic Traits in Canadian Children

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Background: The etiology of autism spectrum disorder is poorly understood. Few studies have investigated the link between endocrine-disrupting chemicals and autistic traits. We examined the relationship between gestational phthalates and autistic traits in 3- to 4-y-old Canadian children. We also investigated potential effect modification by sex and folic acid supplementation.

Methods: We enrolled 2,001 women >18 years of age during the first trimester of pregnancy between 2008 and 2011 from 10 cities in Canada. At 3–4 years of age, 610 children underwent neuropsychological assessments including the Social Responsiveness Scale–II (SRS-2) as a measure of autistic traits and social impairment. We measured 11 phthalate metabolites in maternal first trimester urine samples and assessed folic acid supplementation from reported intakes. We estimated covariate-adjusted differences in SRS-2 *T*-scores with a doubling in phthalate concentrations in 510 children with complete data.

Results: Mean total SRS *T*-score was 45.3 (SD = 6.1). Children with higher gestational exposure to mono-*n*-butyl (MBP) and mono-3-carboxypropyl (MCPP) concentrations exhibited significantly higher total SRS *T*-scores, indicating greater overall social impairment, as well as higher scores on subdomains, indicating deficits in social cognition, social communication, social motivation, and restricted interests/repetitive behaviors. A doubling in MBP or MCPP concentrations was associated with 0.6 (95% CI: 0.1, 1.0) and 0.5 (95% CI: 0.1, 0.8) higher total SRS *T*-scores. Associations were consistently and significantly stronger in boys ($\beta_{MBP} = 1.0$; 95% CI: 0.4, 1.6; $n = 252$) compared with girls ($\beta_{MBP} = 0.1$; 95% CI: −0.6, 0.7; $n = 258$) and among children who had lower prenatal folic acid supplementation (<400 μg/d) ($\beta_{MBP} = 1.3$; 95% CI: 0.4, 2.3; $n = 59$) compared with those who had adequate folic acid supplementation (≥400 μg/d) ($\beta_{MBP} = 0.4$; 95% CI: −0.1, 0.8; $n = 451$).

Conclusions: Higher gestational concentrations of some phthalate metabolites were associated with higher scores of autistic traits as measured by the SRS-2 in boys, but not girls; these small size effects were mitigated by first trimester-of-pregnancy folic acid supplementation. https://doi.org/10.1289/EHP5621

Background

Autism Spectrum Disorder (ASD), which affects about 1.7% of children (Baio et al. 2018), comprises a complex group of brain-based disorders characterized by social deficits, atypical communication, and repetitive and restrictive behaviors (American Psychiatric Association 2013). ASD undoubtedly has an underlying genetic basis, but the incomplete concordance in studies of monozygotic twins indicates a significant role of environmental factors (Gardener et al. 2009; Hallmayer et al. 2011; Sandin et al. 2017). Indeed, the rapid rise in prevalence over the past three decades cannot be explained solely by genetic factors (Landrigan 2010). As such, recent research has focused on understanding environmental factors, such as exposures to neurotoxic chemicals during early brain development, involved in the etiology of ASD (Becerra et al. 2013; Cheslack-Postava et al. 2013; Landrigan 2010; Lyall et al. 2014b; Miodovnik et al. 2011; Pelch et al. 2019; Volk et al. 2013; von Ehrenstein et al. 2019).

Phthalates are a class of multifunctional chemicals with endocrine-disrupting properties (Zota et al. 2014). Two previous studies suggest that phthalate exposures are associated with ASD or its associated traits. In one study of 4,779 children, those who lived in homes with polyvinyl chloride flooring, a significant source of phthalates, were more likely to be diagnosed with ASD (Larsson et al. 2009). Another study reported associations between higher third trimester gestational urinary low-molecular weight phthalate metabolite concentrations and higher Social Responsiveness Scale–II (SRS-2) *T*-scores in 137 seven- to nine-year-old children (Miodovnik et al. 2011). In contrast, three other studies did not observe associations of phthalates in second and third trimester maternal urine (Braun et al. 2014; Shin et al. 2018) or house dust collected 2–5 y after birth (Philippat et al. 2015) with autistic traits or ASD diagnosis. However, one of those studies reported potential effect modification of the association between some phthalates and ASD by prenatal vitamin use (Shin et al. 2018). In addition, two recent studies suggested that folic acid (FA) may mitigate the potential adverse health effects of air pollutants and pesticides in relation to ASD (Goodrich et al. 2018; Schmidt et al. 2017). It is not clear how FA supplementation

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Supplemental Material is available online (https://doi.org/10.1289/EHP5621).

The authors declare they have no actual or potential competing financial interests.

Received 15 May 2019; Revised 15 January 2020; Accepted 17 January 2020; Published 19 February 2020.

may protect from potential effects of environmental pollutants. However, some evidence points to DNA methylation as a mechanistic pathway explaining these observed modification effects because folate serves as a primary methyl-donor and phthalates may induce methylation changes in several regions (Ponsonby et al. 2016; Singh and Li 2012).

In the present study, we examined the relationship between first trimester gestational urinary phthalate concentrations and the development of children's autistic traits as measured by the SRS-2 at 3–4 years of age in a multisite Canadian prospective pregnancy cohort. We also examined effect modification by child sex because phthalates are suspected to have sexually dimorphic neurodevelopmental effects (Ejaredar et al. 2015; Zhang et al. 2019). Finally, we investigated effect modification by gestational FA supplementation to examine whether previously reported findings for pesticides and air pollutants extend to phthalates.

Methods

Study Population

The Maternal–Infant Research on Environmental Chemicals (MIREC) Study is a longitudinal pregnancy cohort study conducted in Canada. Women >18 years of age ($n = 2{,}001$; 1,983 with final consent, and 1,910 singleton live births; see Figure S1) were recruited during the first trimester of pregnancy (6 to <14 weeks gestation), between 2008 and 2011 at 11 sites in 10 Canadian cities. Women who had a medical history of major chronic disease, threatened abortion, or illicit drug use were excluded from the study. Further details regarding inclusion and exclusion criteria have been previously reported (Arbuckle et al. 2013). We collected information from questionnaires and medical charts as well as maternal blood and urine specimens during pregnancy and at delivery. At 36–48 months of age, a convenience subset of 610 children was selected from seven study sites (six cities) to undergo a thorough assessment of their neurodevelopmental status (Braun et al. 2017). Overall, baseline maternal covariates were not substantially different among children with and without neurodevelopmental assessment. Mothers of followed-up children were more likely to be white, to be nonsmokers during pregnancy, and to have lower gestational phthalates concentrations as compared with mothers of children recruited at baseline from the same study sites. There was also a higher proportion of girls in the 3-y follow-up compared with the initially recruited population. Comparisons of mothers who were enrolled at baseline from the seven study sites (six cities) with mothers of children who completed SRS measurements at the 3-y follow-up are presented in Table S1.

All participants were informed of the purpose of the study and gave their written consent to participate in the study. The study was approved by research ethics committees at Health Canada and all the study centers.

Urine Collection and Phthalate Analysis

First trimester urine samples were collected in 125-mL Nalgene® containers (Thermo-Fisher Scientific), aliquoted into 30-mL Nalgene® containers, frozen at −20°C within 2 h of collection, and shipped on dry ice to the MIREC coordinating center in Montreal, where they were stored at −30°C. Concentrations of 11 phthalate monoester metabolites were quantified in first trimester urine samples at the Toxicology Centre of the Quebec Institute of Public Health (Institut national de santé publique du Québec) using liquid chromatography–tandem mass spectrometry (LC-MS/MS) with ultra-performance liquid chromatography (UPLC) coupled with MS/MS and Quattro Premier XE following enzymatic deconjugation (Arbuckle et al. 2013, 2014). Phthalate metabolites included mono-ethyl phthalate (MEP), mono-*n*-butyl phthalate (MBP), mono-benzyl phthalate (MBzP), mono-3-carboxypropyl phthalate (MCPP), monocyclohexyl phthalate (MCHP), mono-*n*-octyl phthalate (MOP), mono-isononyl phthalate (MNP), monomethyl phthalate (MMP), and three metabolites of di-(2-ethylhexyl) phthalate (DEHP): monoethylhexyl phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP). Four of the phthalate metabolites (MCHP, MOP, MNP, and MMP), which were detected in fewer than 20% of the urine samples, were not considered in further analyses. MEP, MBP, MBzP, MEHP, MEHHP, and MEOHP were detected in >97% of urine samples, whereas MCPP was detected in 81% of samples. All values below the limit of detection (LOD) were replaced by LOD divided by the square root of 2. Molar concentrations of hydrolytic (i.e., MEHP) and oxidative (i.e., MEHHP and MEOHP) metabolites of DEHP were summed to create a summary of DEHP concentrations given that individual metabolite concentrations were highly correlated (Pearson $r = 0.91$–0.99). To compare DEHP with other phthalate metabolites expressed in micrograms per liter, the DEHP molar sum was multiplied by the molar weight of MEHP (MW = 278) and expressed as MEHP in micrograms per liter (Wolff et al. 2010). MEP, MBP, MBzP, and MCPP were analyzed separately.

To account for urine dilution, we standardized concentrations of phthalate metabolites for urinary specific gravity (SG) according to the following formula (Arbuckle et al. 2014):

$$P_c = P_i[(SG_m - 1)/(SG_i - 1)]$$

where P_c is the SG-standardized metabolite concentration, P_i is the observed metabolite concentration, SG_i is the SG of the ith urine sample and SG_m is the median SG for the cohort (1.012).

Measurement of Autistic Traits

We assessed autistic traits in 601 children using the SRS-2. The SRS-2 is a parent-reported 65-item questionnaire that provides a quantitative measure of autistic traits in children, specifically those related to children's social behavior, repetitive behaviors, and restricted interests (Constantino and Gruber 2005). Previous studies have shown that the SRS is a valid and reliable instrument for assessment of autistic traits in the general population or in clinical settings (Bölte et al. 2011; Constantino and Todd 2003), with high sensitivity (>0.8) for the diagnosis of ASD (Bölte et al. 2011) using a cutoff of 75 for SRS-2 scores. Higher scores indicate greater degrees of social impairment, with total SRS T-scores ≥60 considered indicative of clinically significant deficiencies in reciprocal social behavior, and T-scores ≥75 being consistent with a clinical diagnosis of ASD. The SRS also provides scores for five subscales: Social Awareness, Social Cognition, Social Communication, Social Motivation, and Restricted Interests and Repetitive Behavior as well as two Diagnostic and Statistical Manual of Mental Disorders (DSM)-compatible subscales, allowing comparison with the DSM-5 ASD diagnostic criteria: Social Communication and Restricted Interests/Repetitive Behavior. Complete data on SRS T-scores and phthalate concentrations were available for 556 children.

Prenatal Folic Acid Supplementation

Study participants reported their supplement and medication intakes in the past 30 days at the time of questionnaire administration (first trimester). Participants provided the name and description of the product, the drug or natural product identification number on the bottle (drug identification number or natural

product number), the amount taken each time (number of, e.g., pills or tablets), and the frequency of intake. A supplement user was defined as someone who consumed FA in the form of a multivitamin or single-vitamin supplement. A total of 78 unique vitamin supplements containing FA were identified by the participants. The FA content and recommended daily intake for each product was obtained from the Health Canada Licensed Natural Health Products Database. For those products not found in the database, the FA content and recommended daily intake was identified on the manufacturer's website. If the participant indicated a brand but not the specific product, the mean FA content for all prenatal supplements from that manufacturer was used. For vitamin supplements that were not found in the Licensed Natural Health Products Database, or the manufacturer's website could not be found, the mean FA content for all supplement products identified in the sample were used to estimate the total daily FA intake. When the participant did not indicate the frequency or number of pills consumed, the daily intake recommended by the manufacturer was assumed. To calculate total daily FA intake from supplements for each participant, the FA content from all vitamin supplements was summed. The doses of total daily FA intake from supplements were divided into three groups: <400 μg, 400–999 μg, and ≥1,000 μg. The recommended FA intake from supplements for women who could become pregnant or are pregnant or lactating is 400 μg/d (IOM 1998; Crider et al. 2018; Page et al. 2017).

Covariates and Potential Confounders

We collected data on sociodemographic factors, lifestyle factors, and medical history during pregnancy and at delivery via questionnaires administered in the first and third trimesters and at delivery as well as medical chart abstractions. At the 3- to 4-y follow-up visit, we also assessed duration of exclusive breastfeeding, current maternal stress using the parenting stress index (PSI) (Abidin 1995) and depressive symptoms using the Center for Epidemiological Studies Depression Scale-10 (CES-D10) (Kohout et al. 1993). All covariates to be included in the models were chosen *a priori* with no reliance on statistical significance. The following variables were considered for inclusion in the multivariable models: child's age at neuropsychological assessment (in months), child sex (male; female), birth weight (grams), maternal age at start of pregnancy (years), parity (no previous child; 1 previous child; 2 or more previous children), maternal education (high school diploma or lower; some college/college or trade school diploma; undergraduate university degree; graduate university degree and higher), household income (≤50,000; 50,001–80,000; 80,001–100,000; >100,000 CAD$), marital status (married or living with a partner; not married or living alone), mother's country of birth [born in North America (Canada and the United States); born elsewhere], race/ethnicity (white; nonwhite), parenting stress index, and depression scores (continuous), first trimester FA supplementation (<400 μg/d; 400–999 μg/d; ≥1,000 μg/d), first trimester blood lead concentrations, alcohol consumption during pregnancy (no; yes), smoking during pregnancy (no; yes), city (Vancouver; Toronto; Hamilton; Kingston; Montreal; Halifax), and year of enrollment (based on first visit date). Maternal stress, depressive symptoms, Home Observation and Measurement of the Environment (HOME) scores (Caldwell and Bradley 1979), and duration of exclusive breastfeeding (in months) assessed at follow-up were also considered. We used directed acyclic graphs (DAGs) to identify the minimum set of covariates to infer potentially unbiased estimates of the association between phthalates and SRS-2 scores (see Figures S2 and S3). In addition, child sex and FA supplementation were forced in all models as potential predictors of autistic traits and effect modifiers of interest. The main models therefore included the following covariates: child sex, FA supplementation, study site (city), year of enrollment, socioeconomic status as informed by household income, maternal education, marital status, and race/ethnicity, maternal age, and parity. Additional sensitivity analyses were conducted including additional covariates that are predictors of autistic traits with no known association with phthalates and that are unlikely to be predicted by autistic traits.

Statistical Analyses

Complete data on SRS scores and phthalate concentrations were available for 556 children, and 46 children had missing data on covariates included in the models (see Figure S1). The final analytical sample consisted of 510 children in the main models. All SG-standardized phthalate concentrations were $\log_2$-transformed to reduce the influence of outliers. First, we conducted multivariable linear regression analyses, adjusting for the set of covariates inferred from the DAG. The results are presented as the adjusted mean difference (β) with 95% confidence interval (95% CI) for a 2-fold increase in SG-standardized urinary phthalate concentrations. Next, we explored potential nonlinear relationships between phthalate concentrations ($\log_2$-transformed) and SRS total and subscale scores using generalized additive models (GAMs) with penalized smoothing regression splines, and visually inspected plots of the smoothed data. We assessed significance with Wald tests using the Bayesian covariance matrix for the coefficients (Marra and Wood 2012) and departure from linearity by comparing the models with urinary phthalate concentrations introduced as a spline function and as a linear term.

Because previous studies showed potential sexually dimorphic associations between phthalates and neurodevelopmental outcomes (Kim et al. 2011; Shin et al. 2018; Zhang et al. 2019), we examined sex-specific associations by including a product interaction term between child sex and phthalate concentrations. We also examined whether prenatal FA supplementation modified the association between urinary phthalate concentrations and SRS scores using the same approach. For this analysis, we estimated and compared the phthalate–SRS association among women who took <400 μg of FA/d with those taking ≥400 μg FA/d because it is recommended that women of child-bearing age who could become pregnant and women who are pregnant or lactating should consume a multivitamin that contains 400 μg/d (Crider et al. 2018; IOM 1998). Further stratification by child sex was not possible due to the small sample size of the <400-μg/d group.

We conducted a series of sensitivity analyses to assess the robustness of the estimates. First, we addressed potential selection bias due to missing data and attrition by applying stabilized inverse probability weights of censoring to all the models and provided results that were generalizable to the initial target population (Hernán et al. 2004; Narduzzi et al. 2014). The application of weights can alleviate the bias resulting from this nonrandom selection and missingness and, therefore, generalize the results from the subset of the population with complete data and follow-up to the original target population. We considered the entire population of the study ($n = 1{,}910$) and calculated the probability of nonmissing information—including both missing data and participants with no follow-up at 3–4 years of age—using a logistic regression model where the response was the nonmissingness at the 3- to 4-y follow-up ($n = 510$) and the covariates were its possible predictors at baseline, including study center, child sex, family income, maternal age, maternal education, marital status, parity, race/ethnicity, phthalates metabolites concentrations, and FA supplementation. We subsequently derived the weights for

each subject as the inverse of the predicted probability and performed the analyses only on the nonmissing observations using a weighted model. Second, we adjusted for first trimester blood lead concentrations, smoking, and alcohol during pregnancy in addition to variables that might impact SRS scores but not gestational phthalates concentrations: admission at a neonatal intensive care unit, HOME scores (Caldwell and Bradley 1979), and exclusive breastfeeding duration. Third, we conducted a sensitivity analysis adjusting for SG as an independent predictor in the models instead of including SG-standardized phthalate concentrations. Further, we conducted sex-stratified analyses to account for any potential sex-dependent confounding. Differences in the associations between boys and girls were tested by comparing the value of d/SE_d to the standard normal distribution, where d is the difference between the two estimates, and $\mathrm{SE}_d = \sqrt{\mathrm{SE}_1^2 + \mathrm{SE}_2^2}$ is the standard error of the difference (Altman and Bland 2003). Finally, we ran models for effect modification by FA supplementation, categorizing FA supplementation into three categories (<400 μg/d; 400–999 μg/d; ≥1,000 μg/d).

All significance tests were two-sided and the level of significance was set at $p < 0.05$ for main estimates and at $p < 0.10$ for interactions. All statistical analyses were conducted using R (version 3.1.2; R Development Core Team). For the interpretation of findings, we placed emphasis on patterns of associations rather than simply relying on p-values.

Results

Descriptive Statistics

The mean age of the children at the time of the examination was 3.4 y (range 3–4 y), and there were slightly fewer boys than girls (48% vs. 52%). Most of the children had at least one older sibling (56%), and 47% were exclusively breastfed for ≥6 months. The mean age of mothers was 33 y; most of whom were white (89%), employed (85%), married or living with a partner (97%), and well educated, with 67% having at least an undergraduate university degree. Few mothers reported smoking (3%) or drinking regularly (7%) during pregnancy; 81% of mothers had adequate FA supplementation (>400 μg/d) in the first trimester of pregnancy (Table 1).

The mean total SRS score was 45.3 [standard deviation (SD) = 6.1]; 11 children (2%) had total SRS T-scores ≥60, whereas 2 children (0.4%) had total SRS T-scores ≥75. Boys, nonwhite children, children who were exclusively breastfed for <3 months, or children who had no older sibling had significantly higher SRS scores. Children of mothers who reported any of the following characteristics had significantly higher total SRS scores: younger, less educated, lower income, no partner, or reported smoking during pregnancy. Children of mothers with high PSI and CES-D-10 scores also had significantly higher SRS scores (Table 1).

SG-standardized geometric mean (GM) urinary phthalate metabolite concentrations were 12.8, 5.4, 33.1, 0.98, and 18.1 μg/L for MBP, MBzP, MEP, MCPP, and ΣDEHP metabolites, respectively (see Table S2). Overall, SG-standardized phthalate metabolite concentrations did not substantially vary with covariates, although we observed higher levels in younger mothers (except for MCPP and ΣDEHP), mothers of boys, mothers who had less education (except for ΣDEHP), and mothers with inadequate FA supplementation (MCPP and ΣDEHP) (Table 1).

Associations between Gestational Phthalate Concentrations and SRS-2 Scores

In multivariable adjusted models, increasing gestational MBP or MCPP urinary concentrations were significantly associated with higher total SRS T-scores indicative of greater social impairment (Table 2). A 2-fold increase in urinary MBP and MCPP concentrations was associated with increases of 0.6 (95% CI: 0.1, 1.0) and 0.5 (95% CI: 0.1, 0.8) points in total SRS scores, respectively.

For SRS subscales, maternal urinary MBP and MCPP concentrations were associated with higher social cognition, social communication, social motivation, and restricted interests/repetitive behaviors subscales (Table 2). A 2-fold increase in MBP and MCPP concentrations was associated with increases of 0.5 (95% CI: 0.1, 1.0) and 0.6 (95% CI: 0.3, 0.9) points, respectively, on the social communication subscale. Comparable estimates were observed for the other SRS subscales (Table 2), regardless of significance. No associations were observed for MBzP, MEP, and DEHP.

Gestational urinary MCPP concentrations and SRS scores exhibited a linear dose–response relationship (see Figure S4), whereas associations with gestational urinary MBP concentrations showed a nonlinear relationship (Figure 1). For total SRS scores, the association was significant ($p = 0.01$) and departed significantly from linearity ($p = 0.03$), with a steeper slope at SG-standardized urinary MBP concentrations above 32 μg/L.

Overall, associations between gestational urinary phthalate concentrations and SRS scores appeared stronger in boys than girls, with many associations for MBP and MEP exhibiting significant ($p < 0.1$) effect modification by child sex (Figure 2; Table 2). A 2-fold increase in gestational urinary MBP concentrations was associated with 1.0 (95% CI: 0.4, 1.6), 1.1 (95% CI: 0.4, 1.7), 0.9 (95% CI: 0.3, 1.6), and 0.9 (95% CI: 0.2, 1.6) higher Total, Social Cognition, Social Communication, and Restricted Interests and Repetitive Behavior scores among boys (Table 2), respectively, but not among girls [β for Total SRS = 0.1; 95% CI: −0.6, 0.7, Social Cognition = 0.2; 95% CI: −0.5, 0.8, Social Communication = 0.1; 95% CI: −0.6, 0.7, and Restricted Interests and Repetitive Behavior = 0.0; 95% CI: −0.7, 0.7 (p for effect modification <0.07 for all)]. MEP showed mainly null associations in boys and negative associations in girls: A 2-fold increase in urinary MEP concentrations was associated with 0.2 (95% CI: −0.2, 0.5) higher total scores in boys and −0.4 (95% CI: −0.7, 0.0) lower total scores in girls (Table 2). The same pattern for MEP was observed for Social Cognition, Social Communication, and Social Motivation (p for effect modification <0.05 for all). No pattern of effect modification by sex was observed for MCPP and MBzP. In sex-stratified GAMs, only MBP exhibited a significant nonlinear dose–response relationship in boys (see Figure S5), and all other associations exhibited a linear shape.

FA supplementation during pregnancy consistently and significantly attenuated the positive associations between gestational urinary phthalate concentrations and high SRS scores (Figure 3; see also Table S3). This trend of effect modification was significant for ΣDEHP and MCPP with all SRS subscales and Total scores, and was also significant for MBP with Social Cognition and Total scores, for MBzP with Social Cognition, Restricted Interests and Repetitive Behavior, and Total SRS scores, and for MEP with Social Cognition and Social Awareness (all at $p < 0.1$). For instance, a 2-fold increase in urinary MCPP concentrations was associated with an increase of 1.8 points (95% CI: 1.0, 2.6) in total SRS scores among children whose mothers had taken <400 μg FA/d. In contrast, the association was weaker in children whose mothers had taken ≥400 μg FA/d (β = 0.3; 95% CI: 0.0, 0.7, p for effect modification <0.001). A similar pattern was observed for other SRS subscores and for other urinary phthalate metabolites.

Sensitivity Analyses

The overall pattern of the results was similar in analyses using inverse probability weighting with those drawn from the main

Table 1. Children's total SRS-2 *T*-scores at 3–4 years of age and maternal SG-standardized urinary phthalate concentrations (μg/L) during pregnancy in relation to covariates (MIREC; $n = 556$).

		Total SRS *T*-score	MBP ($n = 556$)	MBzP ($n = 555$)	MEP ($n = 556$)	MCPP ($n = 556$)	ΣDEHP ($n = 552$)
Characteristics	*n* (556)	Mean (SE)	GM (GSE)	GM (GSE)	GM (GSE)	GM (GSE)	GM (GSE)
Total	556	45.3 (0.3)	12.8 (0.5)	5.4 (0.2)	33.1 (2.0)	0.98 (0.04)	18.1 (0.6)
Child sex							
Boys	268	46.5 (0.5)	14.2 (0.7)	5.8 (0.3)	36.3 (3.1)	1.06 (0.08)	19.4 (0.9)
Girls	288	44.1 (0.3)	11.5 (0.5)	5.1 (0.3)	30.4 (2.5)	0.90 (0.05)	17.0 (0.7)
Maternal age							
≤30	187	46.3 (0.4)	14.3 (0.9)	6.4 (0.4)	35.6 (3.5)	0.96 (0.07)	17.6 (1.0)
31–35	211	44.9 (0.4)	11.9 (0.6)	5.1 (0.3)	32.8 (3.2)	0.99 (0.07)	18.2 (0.9)
≥36	158	44.5 (0.5)	12.2 (0.7)	4.8 (0.3)	30.7 (3.6)	0.97 (0.09)	18.7 (1.1)
Maternal Education							
High school diploma or lower	29	47.4 (1.3)	15.4 (2.3)	6.3 (1.0)	37.6 (11.4)	1.48 (0.33)	19.9 (2.5)
College or trade school diploma	151	46.1 (0.5)	12.3 (0.9)	5.2 (0.4)	40.2 (4.6)	1.07 (0.09)	15.9 (1.0)
Undergraduate university degree	218	45.3 (0.4)	12.5 (0.7)	5.5 (0.3)	31.2 (3.0)	0.93 (0.07)	17.9 (0.8)
Graduate university degree	156	43.9 (0.4)	12.9 (0.8)	5.4 (0.4)	29.5 (3.2)	0.87 (0.07)	20.5 (1.3)
Missing	2	—	—	—	—	—	—
Household income							
≤50,000 CAN$	79	47.9 (0.8)	14.6 (1.5)	6.1 (0.6)	35.3 (5.7)	0.97 (0.11)	16.7 (1.3)
50,001–80,000 CAN$	131	45.7 (0.5)	12.9 (0.9)	6.3 (0.6)	36.7 (4.7)	1.11 (0.09)	17.0 (1.0)
80,001–100,000 CAN$	112	45.2 (0.6)	12.8 (1.0)	5.3 (0.4)	24.4 (2.9)	0.87 (0.10)	18.8 (1.3)
>100,000 CAN$	215	44.1 (0.4)	12.4 (0.7)	4.9 (0.3)	34.7 (3.4)	0.98 (0.07)	19.4 (1.0)
Missing	19	—	—	—	—	—	—
Maternal employment							
No	70	46.4 (0.9)	13.0 (1.6)	5.1 (0.6)	31.6 (5.2)	0.99 (0.13)	18.0 (1.7)
Yes	474	45.0 (0.3)	12.6 (0.5)	5.4 (0.2)	33.3 (2.2)	0.97 (0.05)	18.1 (0.6)
Missing	12	—	—	—	—	—	—
Marital status							
Currently living with a partner	539	45.1 (0.3)	12.8 (0.5)	5.5 (0.2)	32.7 (2.0)	0.98 (0.05)	18.3 (0.6)
Not living with a partner	17	49.2 (1.8)	11.8 (2.4)	4.2 (0.6)	47.4 (15.4)	0.89 (0.17)	12.4 (1.5)
Race/ethnicity							
White	494	45.1 (0.3)	12.6 (0.5)	5.5 (0.2)	31.2 (1.9)	0.97 (0.05)	17.8 (0.6)
Nonwhite	62	46.6 (0.7)	14.5 (1.8)	4.7 (0.5)	52.9 (10.8)	1.00 (0.14)	20.6 (1.8)
Parity							
0	242	46.0 (0.4)	12.5 (0.7)	5.5 (0.4)	38.9 (3.4)	0.89 (0.06)	17.7 (0.9)
1	231	44.6 (0.4)	12.8 (0.7)	5.2 (0.3)	28.9 (2.6)	1.06 (0.07)	18.5 (0.9)
≥2	83	45.1 (0.8)	13.5 (1.1)	5.7 (0.5)	30.2 (5.2)	1.00 (0.11)	18.1 (1.4)
1st trimester FA supplementation							
<400 μg/d	59	45.8 (0.9)	13.3 (1.2)	5.2 (0.5)	35.2 (5.5)	1.07 (0.13)	19.5 (1.7)
400–999 μg/d	108	45.9 (0.6)	10.7 (0.8)	4.8 (0.4)	29.1 (3.2)	0.70 (0.07)	15.1 (1.1)
≥1,000 μg/d	344	44.9 (0.3)	13.1 (0.6)	5.6 (0.3)	34.7 (2.8)	1.06 (0.06)	18.6 (0.8)
Missing	45	—	—	—	—	—	—
Smoking during pregnancy							
No	540	45.2 (0.3)	12.9 (0.5)	5.5 (0.2)	33.1 (2.0)	0.98 (0.05)	18.3 (0.6)
Yes	16	48.6 (1.8)	8.2 (1.8)	3.9 (1.2)	31.9 (7.5)	0.83 (0.18)	11.8 (2.6)
Alcohol during pregnancy							
Never	313	45.1 (0.3)	12.6 (0.6)	5.4 (0.3)	32.6 (2.6)	0.96 (0.06)	17.7 (0.8)
<1 drink/week	203	45.3 (0.4)	13.5 (0.8)	5.6 (0.3)	35.8 (3.5)	1.03 (0.07)	18.8 (0.9)
≥1 drink/week	39	46.1 (1.0)	10.3 (0.9)	4.8 (0.5)	25.9 (5.7)	0.88 (0.18)	17.8 (1.6)
Missing	1	—	—	—	—	—	—
Duration of exclusive breastfeeding							
<3 months	170	46.5 (0.5)	12.7 (0.8)	5.3 (0.4)	36.5 (3.9)	0.95 (0.07)	18.4 (1.1)
3–6 months	111	44.4 (0.6)	12.1 (0.8)	5.5 (0.4)	38.0 (5.4)	0.88 (0.09)	18.5 (1.3)
≥6 months	263	44.8 (0.3)	13.2 (0.7)	5.5 (0.3)	30.0 (2.6)	1.04 (0.07)	17.8 (0.8)
Missing	12	—	—	—	—	—	—
Parenting stress index scores							
<23	485	44.4 (0.2)	12.5 (0.5)	5.3 (0.2)	33.7 (2.2)	0.98 (0.05)	18.1 (0.6)
≥23	58	51.6 (1.2)	14.9 (1.9)	6.4 (0.9)	27.6 (4.0)	0.93 (0.15)	18.0 (1.3)
Missing	13	—	—	—	—	—	—
Maternal CES-D10 Score							
<10	454	44.8 (0.3)	12.7 (0.5)	5.4 (0.2)	32.4 (2.1)	0.98 (0.05)	17.8 (0.6)
≥10	84	47.2 (0.6)	12.8 (1.2)	5.4 (0.6)	35.8 (5.7)	0.91 (0.10)	19.0 (1.5)
Missing	18	—	—	—	—	—	—

Note: —, no data; CES-D10, Center for Epidemiological Studies Depression Scale-10; DEHP, di-(2-ethylhexyl) phthalate; FA, folic acid; GM, geometric mean; GSE, geometric standard error; MBP, mono-*n*-butyl phthalate; MBzP, mono-benzyl phthalate; MCPP, mono-3-carboxypropyl phthalate; MEP, mono-ethyl phthalate; MIREC, Maternal–Infant Research on Environmental Chemicals (study); SE, standard error; SG, specific gravity; SRS-2, Social Responsiveness Scale–II.

analyses (Table 3; see also Figure S6). Additional adjustment for smoking and alcohol during pregnancy, first trimester blood lead concentrations, admission at a neonatal intensive care unit, HOME Inventory scores, and exclusive breastfeeding duration did not appreciably alter the results (see Table S4). Categorizing FA supplementation into three categories yielded similar results, with associations being significantly stronger only in children of women with <400 μg FA/d (see Figure S7). Further, conducting sex-stratified analyses to account for potential sex-dependent confounding did not alter the overall pattern of results (see Table

Table 2. Associations between maternal SG-standardized urinary phthalate concentrations (for a 2-fold increase) and children's SRS-2 *T*-scores at 3–4 years of age (MIREC; $n = 510$).

		All		Boys	Girls	
SRS subscale	Phthalate	β (95% CI)	*p*-Value	β (95% CI)	β (95% CI)	*p*-EM
Total SRS *T*-score	MBP	0.6 (0.1, 1.0)	0.02	1.0 (0.4, 1.6)	0.1 (−0.6, 0.7)	0.03
	MBzP	0.2 (−0.2, 0.6)	0.42	0.3 (−0.3, 0.8)	0.1 (−0.5, 0.6)	0.60
	MEP	−0.1 (−0.4, 0.1)	0.34	0.2 (−0.2, 0.5)	−0.4 (−0.7, 0.0)	0.04
	MCPP	0.5 (0.1, 0.8)	0.01	0.6 (0.1, 1.0)	0.3 (−0.2, 0.9)	0.50
	ΣDEHP	0.1 (−0.4, 0.6)	0.74	0.4 (−0.3, 1.1)	−0.3 (−1.0, 0.4)	0.17
Social Awareness	MBP	0.3 (−0.3, 0.9)	0.29	0.7 (−0.1, 1.5)	−0.1 (−0.9, 0.7)	0.17
	MBzP	0.4 (−0.1, 0.9)	0.14	0.5 (−0.2, 1.2)	0.3 (−0.4, 1.0)	0.67
	MEP	−0.1 (−0.4, 0.3)	0.75	−0.2 (−0.7, 0.3)	0.1 (−0.4, 0.5)	0.53
	MCPP	0.2 (−0.3, 0.6)	0.42	0.4 (−0.2, 0.9)	−0.1 (−0.7, 0.6)	0.38
	ΣDEHP	−0.4 (−1.0, 0.3)	0.25	−0.2 (−1.1, 0.7)	−0.5 (−1.5, 0.4)	0.66
Social Cognition	MBP	0.6 (0.2, 1.1)	0.01	1.1 (0.4, 1.7)	0.2 (−0.5, 0.8)	0.05
	MBzP	0.2 (−0.2, 0.6)	0.34	0.1 (−0.5, 0.7)	0.3 (−0.3, 0.8)	0.69
	MEP	−0.1 (−0.4, 0.2)	0.58	0.2 (−0.2, 0.6)	−0.4 (−0.7, 0.0)	0.03
	MCPP	0.3 (0.0, 0.7)	0.06	0.5 (0.1, 1.0)	0.1 (−0.5, 0.6)	0.22
	ΣDEHP	0.1 (−0.4, 0.6)	0.72	0.5 (−0.2, 1.2)	−0.3 (−1.1, 0.4)	0.13
Social Communication	MBP	0.5 (0.1, 1.0)	0.02	0.9 (0.3, 1.6)	0.1 (−0.6, 0.7)	0.05
	MBzP	0.0 (−0.4, 0.4)	0.95	0.1 (−0.5, 0.6)	0.0 (−0.6, 0.5)	0.83
	MEP	−0.1 (−0.4, 0.2)	0.45	0.2 (−0.2, 0.6)	−0.4 (−0.8, 0.0)	0.02
	MCPP	0.6 (0.3, 0.9)	<0.001	0.7 (0.2, 1.1)	0.5 (0.0, 1.0)	0.67
	ΣDEHP	0.2 (−0.3, 0.8)	0.35	0.5 (−0.2, 1.2)	−0.1 (−0.8, 0.6)	0.21
Social Motivation	MBP	0.5 (0.0, 1.0)	0.06	0.8 (0.1, 1.6)	0.2 (−0.6, 0.9)	0.21
	MBzP	0.1 (−0.4, 0.6)	0.66	0.1 (−0.6, 0.8)	0.1 (−0.6, 0.7)	0.94
	MEP	−0.3 (−0.6, 0.1)	0.10	0.1 (−0.4, 0.5)	−0.6 (−1.0, −0.1)	0.04
	MCPP	0.4 (0.0, 0.8)	0.07	0.4 (−0.2, 0.9)	0.4 (−0.3, 1.0)	0.98
	ΣDEHP	0.0 (−0.6, 0.6)	0.99	0.3 (−0.5, 1.1)	−0.3 (−1.2, 0.5)	0.31
Restricted Interests/Repetitive Behavior	MBP	0.5 (0.0, 1.0)	0.05	0.9 (0.2, 1.6)	0.0 (−0.7, 0.7)	0.07
	MBzP	0.3 (−0.2, 0.7)	0.27	0.5 (−0.1, 1.2)	0.0 (−0.6, 0.6)	0.21
	MEP	−0.1 (−0.4, 0.2)	0.73	0.2 (−0.2, 0.6)	−0.3 (−0.7, 0.1)	0.13
	MCPP	0.5 (0.1, 0.9)	0.01	0.6 (0.1, 1.1)	0.4 (−0.2, 1.0)	0.70
	ΣDEHP	0.2 (−0.3, 0.8)	0.44	0.4 (−0.4, 1.2)	0 (−0.8, 0.8)	0.53
DSM-5–compatible Social Communication	MBP	0.6 (0.1, 1.0)	0.01	1.0 (0.4, 1.6)	0.1 (−0.5, 0.7)	0.03
	MBzP	0.1 (−0.3, 0.5)	0.5	0.2 (−0.4, 0.7)	0.1 (−0.4, 0.7)	0.90
	MEP	−0.2 (−0.4, 0.1)	0.27	0.1 (−0.2, 0.5)	−0.4 (−0.8, 0.0)	0.04
	MCPP	0.5 (0.1, 0.8)	0.01	0.6 (0.1, 1.0)	0.3 (−0.2, 0.8)	0.41
	ΣDEHP	0.1 (−0.4, 0.6)	0.73	0.4 (−0.3, 1.1)	−0.3 (−1.0, 0.5)	0.18
DSM-5–compatible Restricted Interests/Repetitive Behavior	MBP	0.5 (0.0, 1.0)	0.05	0.9 (0.2, 1.6)	0.0 (−0.7, 0.7)	0.07
	MBzP	0.3 (−0.2, 0.7)	0.27	0.5 (−0.1, 1.2)	0.0 (−0.6, 0.6)	0.21
	MEP	−0.1 (−0.4, 0.2)	0.72	0.2 (−0.2, 0.6)	−0.3 (−0.7, 0.1)	0.13
	MCPP	0.5 (0.1, 0.9)	0.01	0.6 (0.1, 1.1)	0.4 (−0.2, 1.0)	0.69
	ΣDEHP	0.2 (−0.4, 0.8)	0.45	0.4 (−0.4, 1.2)	0.0 (−0.8, 0.8)	0.53

Note: All models were adjusted for child sex, folic acid supplementation, study site (city), year of enrollment, household income, maternal education, marital status, race/ethnicity, maternal age, and parity. Corresponding estimates and 95% CI are provided in Figure 2. CI, confidence interval; DEHP, di-(2-ethylhexyl) phthalate; MBP, mono-*n*-butyl phthalate; MBzP, mono-benzyl phthalate; MCPP, mono-3-carboxypropyl phthalate; MEP, mono-ethyl phthalate; MIREC, Maternal–Infant Research on Environmental Chemicals (study); *p*-EM, effect *p*-value for the interaction term between phthalates concentrations and sex in the models; SG, specific gravity; SRS-2, Social Responsiveness Scale–II; ΣDEHP, sum of the metabolites of DEHP.

S5). Finally, including SG as a covariate in the models instead of including SG-standardized phthalate concentrations yielded similar findings (see Table S6).

Discussion

In this prospective Canadian pregnancy cohort, we found that first trimester gestational urinary concentrations of MBP and MCPP phthalate metabolites were associated with poorer reciprocal social behavior in 3- to 4-y-old children, as indicated by higher scores on the Social Communication subscales of the SRS-2. These associations showed a consistent sex-specific pattern; associations of maternal urinary phthalate concentrations with SRS-2 *T*-scores were stronger in boys, with even opposite directions (positive in boys and negative in girls) for the associations between MEP and SRS-2 *T*-scores. Moreover, we found that associations between phthalate concentrations and SRS-2 scores were stronger among children whose mothers had lower FA supplementation during pregnancy (<400 μg/d). These findings add to a growing body of evidence showing that gestational exposure to potential neurotoxic chemicals, such as phthalates, could play a role in the development of ASD and its related traits (Miodovnik et al. 2011; Schmidt et al. 2017; von Ehrenstein et al. 2019).

Concentrations of phthalate metabolites in our study were comparable to those reported for women 20–39 years of age in Cycle 2 of the Canadian Health Measures Survey 2009–2011 (CHMS) (Health Canada 2013). In comparison with gestational urinary concentrations in The Infant Development and Environment Study (TIDES) (2010–2012) (Swan et al. 2015), median concentrations of MBP, MBzP, and DEHP were slightly higher in the MIREC study, whereas median concentrations of MEP were similar. In addition, median concentrations of phthalates in our study were lower for MBP, MEP, MCPP, and DEHP and higher for MBzP in comparison with the levels reported in a cohort study of pregnant Puerto Rican women [Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) 2010–2012] (Cantonwine et al. 2014). In comparison with the Taiwanese Tainan birth cohort (2013–2014), median concentrations of MEP and MBzP were higher in the MIREC study, whereas median concentrations of DEHP and MBP were similar (Huang et al. 2016). Finally, measured phthalates concentrations tended

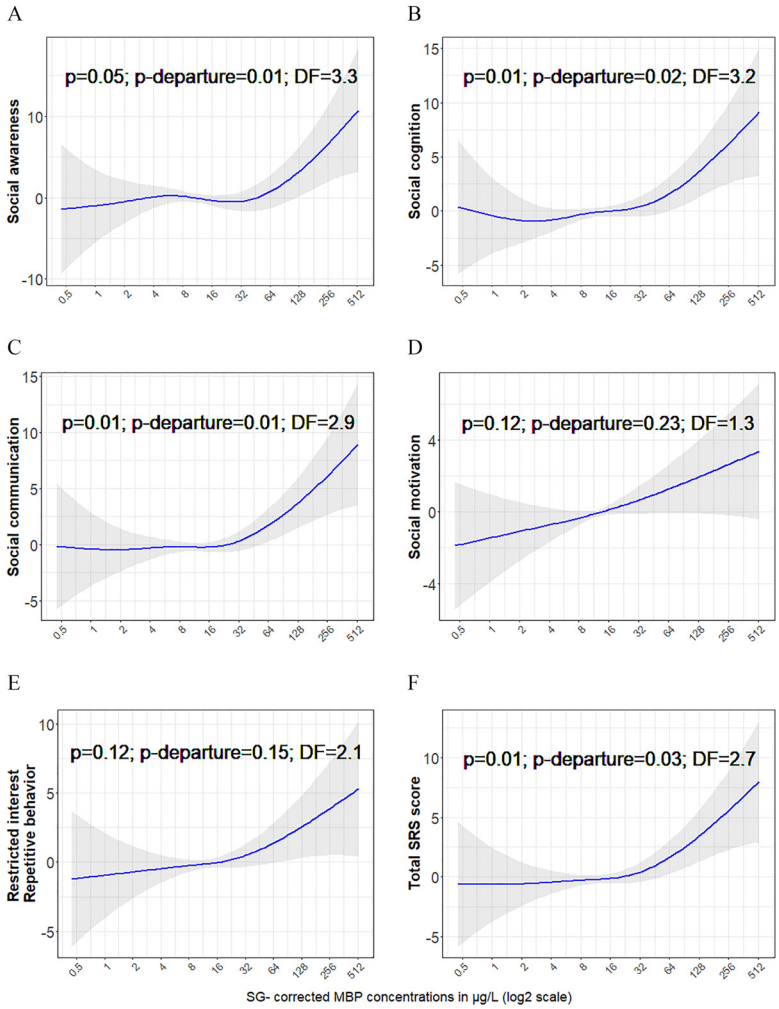

Figure 1. Exposure–response relationship and 95% confidence intervals for the associations between maternal SG-standardized urinary MBP concentrations (in micrograms per liter) and child SRS-2 *T*-scores at 3–4 years of age ($n = 510$). (A) Social Awareness; (B) Social Cognition; (C) Social Communication; (D) Social Motivation; (E) Restricted Interest/Repetitive Behavior; and (F) Total SRS score. *p*-Values for significance (*p*) were derived with Wald tests using the Bayesian covariance matrix for the coefficients and departure from linearity (*p*-departure) were derived by comparing the models with urinary phthalate concentrations introduced as a spline function and as a linear term. Note: DF, degrees of freedom for the smooth term; MBP, mono-*n*-butyl; SG, specific gravity; SRS-2, Social Responsiveness Scale–II.

to be lower than measured concentrations in pregnant women from earlier cohorts from the United States and Spain (Casas et al. 2011; Engel et al. 2009).

Maternal FA consumption during pregnancy has been shown to be associated with reduced risk of ASD (DeVilbiss et al. 2015; Iglesias Vázquez et al. 2019; Wang et al. 2017). In the present study, we also observed that adequate supplementation attenuated the potential harmful effects of phthalates. To our knowledge, this is the first study to report on these potential protective effects of folate supplementation in regard to the relationship between phthalates exposure and autistic traits. Similar effect modification by prenatal vitamin use has been reported for the associations between MCPP and mono-carboxyisooctyl phthalate (MCOP) and nontypical development (Shin et al. 2018). In addition, Shin et al. (2018) reported protective effects of mono-isobutyl phthalate, MCPP, and MCOP in relation to child's ASD risk in mothers who reported prenatal vitamins use, and null associations in children of mothers who reported no prenatal vitamins use. Comparable findings pointing to a potential protective effect of folate against potential effects of chemicals on ASD have also been reported for pesticides and air pollution (Goodrich et al. 2018; Schmidt et al. 2017).

Folate metabolism is required for the biosynthesis of nucleotides and is also involved in the synthesis of essential phospholipids and neurotransmitters. Reduced folate intake can also reduce the availability of cellular methyl donors, which, during critical windows of development, could interfere with the production of these neurodevelopmental factors (Miller 2008). Indeed, low folate status is associated with decreases in global DNA methylation in humans (Crider et al. 2012), and dietary deficiency of methyl donors has been associated with DNA hypomethylation in the brains of rats and in genes controlling brain development in rat fetuses (Christman et al. 1993; DeVilbiss et al. 2015; Niculescu et al. 2006).

Phthalates, which are established endocrine disruptors, could affect the developing brain by altering maternal or fetal thyroid function (Huang et al. 2007; Johns et al. 2015) or by reducing the production of fetal androgens (Howdeshell et al. 2008). Both of these hormonal systems are critical for early brain development (Hiort 2013; Morreale de Escobar et al. 2004). Reduction of testosterone production by phthalates in the male fetus is a potential mechanism explaining the reported sex-specific associations. Experimental data have established that many phthalate metabolites are potent at inhibiting testosterone production in male rats

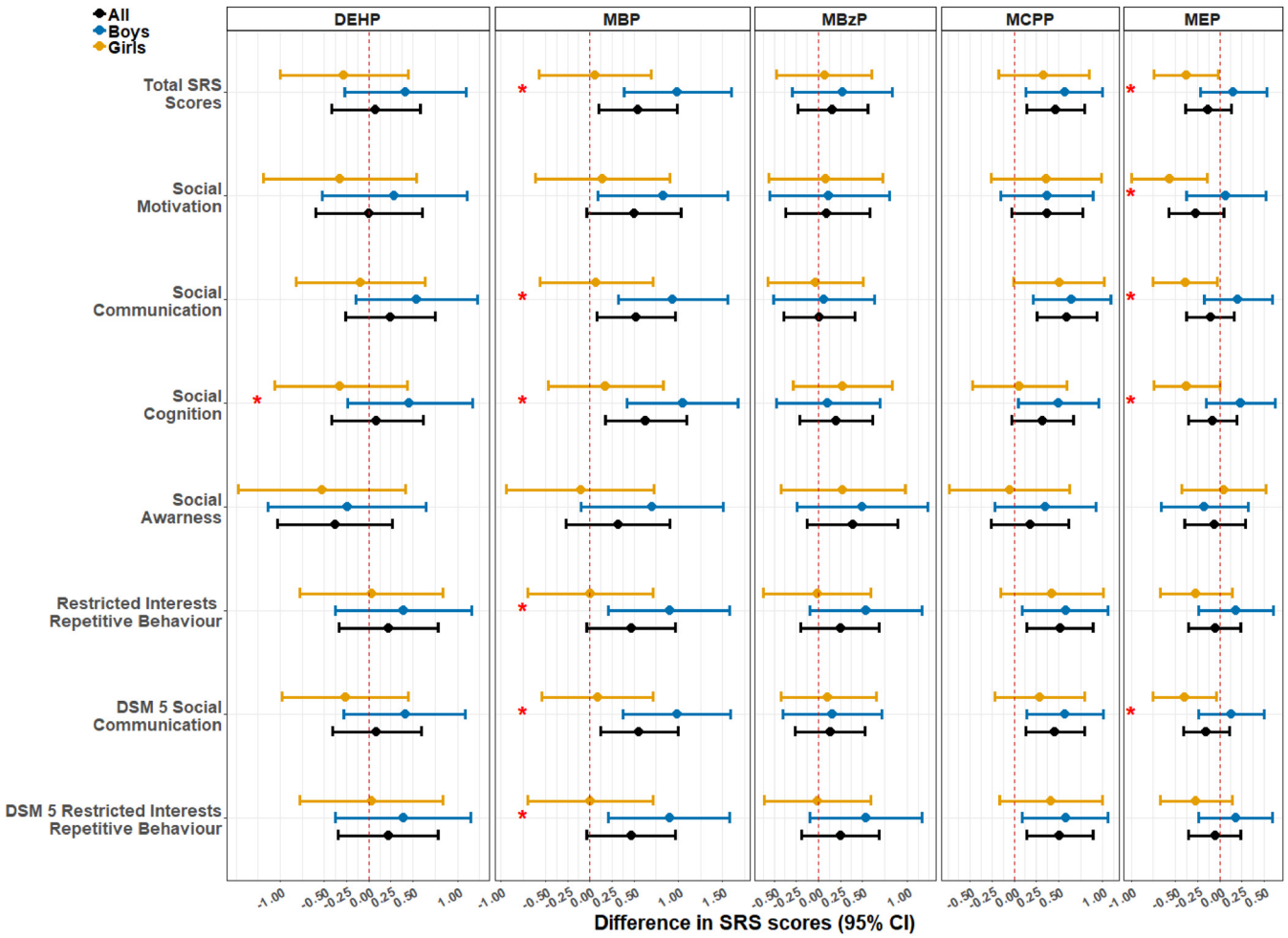

Figure 2. Difference (95% CI) in children's SRS-2 *T*-scores for a doubling in gestational SG-standardized urinary phthalate concentrations, by child sex ($n = 510$). Significant effect modification by sex at $p < 0.1$ is indicated by red asterisks. Corresponding estimates and *p*-values for effect modification are provided in Table 2. Note: BP, mono-*n*-butyl phthalate; CI, confidence interval; DEHP, di-(2-ethylhexyl) phthalate; DSM-5, Diagnostic and Statistical Manual of Mental Disorders–5th edition; MBzP, mono-benzyl phthalate; MCPP, mono-3-carboxypropyl phthalate; MEP mono-ethyl phthalate; SG, specific gravity; SRS-2, Social Responsiveness Scale–II.

during fetal development (Howdeshell et al. 2008). In humans, a recent systematic review based on exposure levels in the general population reported that all but MEP had moderate or greater evidence of male reproductive effects, including decreased testosterone levels (Radke et al. 2018). Phthalate's sex-specific effects on a range of neurodevelopmental outcomes were also shown in human studies as well, with stronger evidence for potential neurobehavioral effects in boys, especially for MBP (reviewed by Ejaredar et al. 2015; Zhang et al. 2019).

Phthalates have also been shown to yield changes in DNA methylation profiles, especially in genes involved in the androgen and estrogen response (Chen et al. 2018), inflammatory response, endocrine function, and male fertility (Solomon et al. 2017). Thus, low folate status and toxicants, such as phthalates, may potentiate each other through common physiologic pathways, including epigenetic and hormonal changes, thus enhancing central nervous system vulnerability (Cory-Slechta et al. 2008; Perera et al. 2013). These findings therefore point to a potential epigenetic mechanism underlying effects of phthalates on brain development that may also operate through alterations of DNA methylation and gene expression in key neurodevelopmental genes (Ponsonby et al. 2016). Our findings also point to a potential role of FA supplementation in overcoming the effects of the phthalates and other neurotoxicants (Goodrich et al. 2018; Schmidt et al. 2017).

There are several limitations of our study. First, we relied on a single urine sample to assess exposure to phthalates during pregnancy. Urinary concentrations of phthalates may vary considerably due to episodic exposures and their short half-lives; serial and/or pooled within-subject measurements are desirable, and previous studies showed that metabolites with relatively consistent exposure sources such as personal care products or indoor residential environments (e.g., MEP and MBP) had relatively high intraclass correlation coefficients, ranging between 0.4 and 0.9 (Braun et al. 2012; Fisher et al. 2015; Shin et al. 2019). However, such a source of bias, if present, is likely nondifferential and would bias our findings toward the null (Pearce et al. 2007). Second, the SRS-2 is not designed to diagnose ASD; however, it has been shown to have high internal validity, reliability, and reproducibility (Lyall et al. 2014a). Scores on the SRS and Autism Diagnostic Interview-Revised (ADI-R), the gold standard instrument for ASD diagnosis, are strongly correlated ($r = 0.7$ for SRS scores and ADI-R algorithm scores for DSM-IV criteria) (Constantino et al. 2003). Further, the SRS was shown to reliably distinguish ASD children from both nonaffected children and those with other neuropsychiatric conditions (Constantino et al.

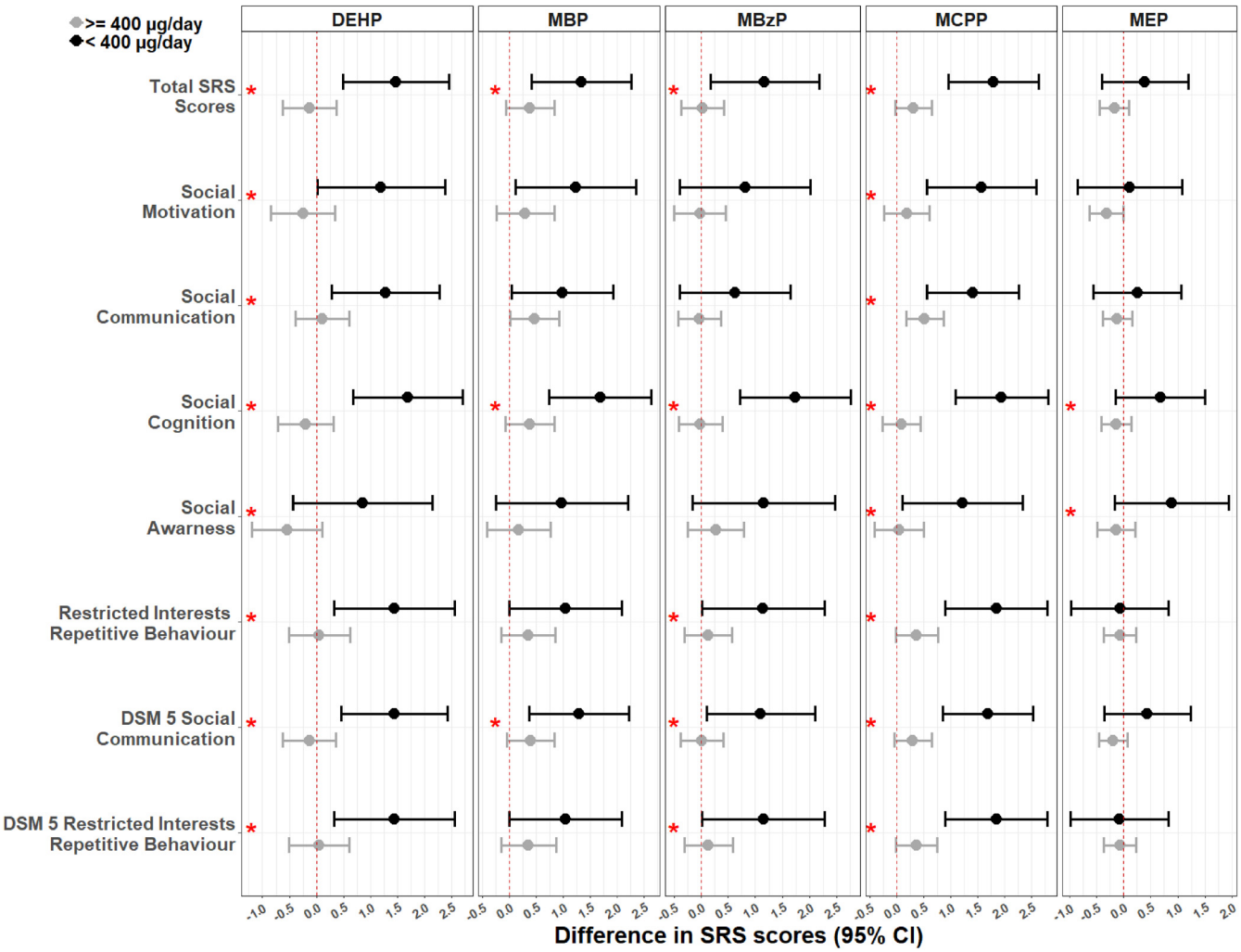

Figure 3. Difference (95% CI) in children's SRS-2 *T*-scores for a doubling in gestational SG-standardized urinary phthalate concentrations, by folic acid supplementation during pregnancy (<400 µg/d/ ≥ 400 µg/d) ($n = 510$). Significant effect modification by folic acid supplementation at $p < 0.1$ is indicated by red asterisks. Corresponding estimates and *p*-values for effect modification are provided in supplementary material, Table S3. Note: CI, confidence interval; DEHP, di-(2-ethylhexyl) phthalate; DSM-5, Diagnostic and Statistical Manual of Mental Disorders–5th edition; MBP, mono-*n*-butyl phthalate; MBzP, monobenzyl phthalate; MCPP, mono-3-carboxypropyl phthalate; MEP mono-ethyl phthalate; SG, specific gravity; SRS-2, Social Responsiveness Scale–II.

2003; Wang et al. 2012), although some studies showed that it lacks specificity for distinguishing children with ASD from those with behavioral disorders such as oppositional and conduct disorders (Bölte et al. 2008; Cholemkery et al. 2014). On the other hand, the SRS allows the capture of subclinical deficits in ASD-related traits, which may be more appropriate in the general population of children. Our findings thus apply across the population and suggest influences on ASD-related traits across the spectrum that extends into the general population. Third, we relied on FA supplementation as a measure of total FA intake and we did not include any measures of dietary sources. However, FA-supplement use has been shown to be the main determinant of folate status in Canadian women of child bearing age (Colapinto et al. 2012), and dietary folate intake was not a predictor of breast-milk folate content in this cohort even in supplement-nonuser women, although the food frequency questionnaire was not validated for folate intake (Page et al. 2017). Other nutrients that may also exert a potential protective role regarding autistic traits and may be correlated with FA intake were not taken into account in the present study. Future investigations with measures of folate and other vitamins in maternal blood are needed to account for any potential confounding. Fourth, our findings may have limited generalizability and may not apply to the general population because the MIREC cohort is largely Caucasian with higher income and education levels compared with the overall Canadian population of women in childbearing age. Fifth, the possibility that other unmeasured health factors—that are predictors of autistic traits and also associated with phthalates concentrations—may explain part of the observed associations cannot be excluded. Finally, it is worth mentioning that the observed effect sizes in the present study were relatively modest. However, the standardized effect of MBP (corresponding to a 1-SD increase) on total SRS scores amounts to an increase of 0.11 SD in all children, 0.20 SD in boys, and 0.21 SD in children of mothers with inadequate FA supplementation. In the context of population health, the impact of a factor at the population level depends not only on the magnitude of its impact on health, or its effect size, but also on the distribution of the factor. Given the widespread and ubiquitous exposure to phthalates, these small effect sizes may have a considerable impact at the population level (Bellinger 2007).

The present study has several notable strengths. First, we used a prospective cohort with participants from six Canadian cities and a larger sample size than used in previous studies. Second, the study was designed primarily with assessment of environmental

Table 3. Associations between maternal SG-standardized urinary phthalate concentrations and children's SRS-2 *T*-scores at 3–4 years of age, using inverse probability censoring weights to adjust for potential selection bias.

		All		Boys	Girls	
SRS subscale	Phthalate	β (95% CI)	*p*-Value	β (95% CI)	β (95% CI)	*p*-EM
Total SRS *T*-score	MBP	0.5 (0.0, 1.0)	0.05	0.9 (0.3, 1.5)	−0.1 (−0.7, 0.6)	0.04
	MBzP	0.2 (−0.2, 0.6)	0.41	0.3 (−0.2, 0.9)	0.0 (−0.6, 0.6)	0.42
	MEP	−0.1 (−0.3, 0.2)	0.64	0.2 (−0.2, 0.6)	−0.4 (−0.8, 0.0)	0.04
	MCPP	0.4 (0.0, 0.7)	0.03	0.5 (0.0, 0.9)	0.2 (−0.3, 0.8)	0.44
	DEHP	0.1 (−0.4, 0.6)	0.78	0.4 (−0.3, 1.1)	−0.3 (−1.1, 0.5)	0.18
Social Awareness	MBP	0.1 (−0.5, 0.7)	0.70	0.4 (−0.4, 1.2)	−0.2 (−1.1, 0.6)	0.28
	MBzP	0.4 (−0.1, 1.0)	0.13	0.6 (−0.2, 1.4)	0.2 (−0.5, 1.0)	0.49
	MEP	0.0 (−0.4, 0.3)	0.81	−0.2 (−0.7, 0.3)	0.1 (−0.4, 0.6)	0.40
	MCPP	0.0 (−0.4, 0.5)	0.85	0.1 (−0.4, 0.7)	−0.1 (−0.8, 0.6)	0.60
	DEHP	−0.4 (−1.1, 0.3)	0.24	−0.4 (−1.3, 0.5)	−0.5 (−1.5, 0.5)	0.89
Social Cognition	MBP	0.6 (0.1, 1.0)	0.03	1.0 (0.4, 1.7)	0.0 (−0.7, 0.7)	0.03
	MBzP	0.2 (−0.2, 0.6)	0.38	0.2 (−0.4, 0.8)	0.2 (−0.4, 0.8)	0.93
	MEP	0.0 (−0.3, 0.3)	0.97	0.3 (−0.1, 0.7)	−0.4 (−0.8, 0.1)	0.03
	MCPP	0.2 (−0.1, 0.6)	0.23	0.4 (−0.1, 0.9)	−0.1 (−0.6, 0.5)	0.22
	DEHP	0.1 (−0.5, 0.6)	0.84	0.4 (−0.3, 1.1)	−0.4 (−1.2, 0.4)	0.14
Social Communication	MBP	0.4 (0.0, 0.9)	0.05	0.9 (0.2, 1.5)	−0.1 (−0.8, 0.6)	0.04
	MBzP	0.0 (−0.5, 0.4)	0.83	0.1 (−0.5, 0.7)	−0.2 (−0.7, 0.4)	0.58
	MEP	0.0 (−0.3, 0.2)	0.73	0.3 (−0.1, 0.6)	−0.4 (−0.8, 0.0)	0.02
	MCPP	0.6 (0.2, 0.9)	<0.001	0.7 (0.2, 1.1)	0.4 (−0.2, 1.0)	0.47
	DEHP	0.3 (−0.2, 0.8)	0.3	0.6 (−0.1, 1.3)	−0.1 (−0.9, 0.7)	0.19
Social Motivation	MBP	0.4 (−0.1, 1.0)	0.14	0.7 (−0.1, 1.4)	0.1 (−0.6, 0.9)	0.35
	MBzP	0.1 (−0.3, 0.6)	0.56	0.2 (−0.5, 0.9)	0.1 (−0.6, 0.8)	0.88
	MEP	−0.2 (−0.5, 0.1)	0.19	0.1 (−0.3, 0.6)	−0.6 (−1.1, −0.1)	0.03
	MCPP	0.2 (−0.2, 0.6)	0.26	0.2 (−0.3, 0.7)	0.3 (−0.4, 0.9)	0.92
	DEHP	0.0 (−0.6, 0.6)	0.90	0.2 (−0.6, 1.0)	−0.4 (−1.3, 0.5)	0.34
Restricted Interests/Repetitive Behavior	MBP	0.4 (−0.1, 1.0)	0.09	0.9 (0.2, 1.5)	0.0 (−0.7, 0.7)	0.09
	MBzP	0.4 (−0.1, 0.8)	0.11	0.7 (0.1, 1.4)	0.0 (−0.6, 0.7)	0.12
	MEP	0.0 (−0.3, 0.3)	0.85	0.2 (−0.2, 0.7)	−0.2 (−0.7, 0.2)	0.13
	MCPP	0.5 (0.1, 0.9)	0.02	0.6 (0.1, 1.1)	0.3 (−0.3, 0.9)	0.46
	DEHP	0.2 (−0.4, 0.8)	0.47	0.4 (−0.4, 1.1)	0.0 (−0.8, 0.9)	0.53
DSM-5–compatible Social Communication	MBP	0.4 (0.0, 0.9)	0.06	0.9 (0.2, 1.5)	0.0 (−0.7, 0.6)	0.05
	MBzP	0.1 (−0.3, 0.5)	0.57	0.2 (−0.4, 0.8)	0.0 (−0.5, 0.6)	0.67
	MEP	−0.1 (−0.4, 0.2)	0.52	0.2 (−0.2, 0.6)	−0.4 (−0.8, 0.0)	0.04
	MCPP	0.4 (0.0, 0.7)	0.04	0.5 (0.0, 0.9)	0.2 (−0.4, 0.7)	0.39
	DEHP	0.1 (−0.4, 0.6)	0.76	0.4 (−0.3, 1.1)	−0.3 (−1.1, 0.5)	0.21
DSM-5–compatible Restricted Interests/Repetitive Behavior	MBP	0.4 (−0.1, 1.0)	0.08	0.9 (0.2, 1.5)	0.0 (−0.7, 0.7)	0.09
	MBzP	0.4 (−0.1, 0.8)	0.11	0.7 (0.1, 1.4)	0.0 (−0.6, 0.7)	0.12
	MEP	0.0 (−0.3, 0.3)	0.85	0.2 (−0.2, 0.7)	−0.2 (−0.7, 0.2)	0.13
	MCPP	0.5 (0.1, 0.8)	0.02	0.6 (0.1, 1.1)	0.3 (−0.3, 0.9)	0.46
	DEHP	0.2 (−0.4, 0.8)	0.48	0.4 (−0.4, 1.1)	0.0 (−0.8, 0.9)	0.53

Note: All models were adjusted for study city, household income, maternal education, maternal age, parity, marital status, race/ethnicity, folic acid supplementation, and year of enrollment. β represents the change in point score for a 2-fold increase in urinary phthalate concentration. CI, confidence interval; DEHP, di-(2-ethylhexyl) phthalate; MBP, mono-*n*-butyl phthalate; MBzP, mono-benzyl phthalate; MCPP, mono-3-carboxypropyl phthalate; MEP mono-ethyl phthalate; *p*-EM, effect *p*-value for the interaction term between phthalates concentrations and sex in the models; SG, specific gravity; SRS-2, Social Responsiveness Scale–II.

contaminants and neurodevelopmental outcomes in mind; thus, biospecimen storage and data collection were optimized for these study goals. Moreover, our use of sensitive and specific biomarkers to assess phthalate exposures and the measurement of phthalates metabolites, rather than the parent phthalate diesters, is more relevant because these metabolites have been shown to be responsible for biological activities attributed to phthalates (Heindel and Powell 1992). Third, both participants and SRS administrators were blinded to exposure status. Finally, we accounted for numerous other potential risk factors for social impairment and ASD, including socioeconomic factors, FA supplementation, and other well-known neurotoxicants such as lead and maternal smoking during pregnancy. The potential influence of any unmeasured confounders appears minimal. Indeed, sensitivity analyses, including home enrichment, exclusive breastfeeding duration, as well as analyses adjusting for potential selection bias from censoring due to loss of follow-up and missing data, did not appear to influence our estimates. Finally, our study opens new avenues in studying some nutritional factors as potential protectors against deleterious effects of environmental exposures. Future studies should extend the scope of these investigations to other nutrients involved in methylation such as vitamins B_{12} and B_6.

Conclusion

The results from the present study suggest that first trimester gestational urinary phthalate exposures may be associated with fetal neurodevelopment. The present study provides new insights regarding the potential neurotoxicity of phthalates, which are widely used in certain plastics, personal care products, pharmaceuticals, food packaging, and medical devices. Moreover, it supports earlier research showing increased susceptibility of the developing brain, especially the male fetal brain, to the impact of toxic chemicals. Finally, these results provide further evidence of the potential opportunities for primary prevention of ASD by reducing exposures to environmental toxicants and ensuring adequate FA supplementation in the periconceptional period for pregnant women.

Acknowledgments

We thank the Maternal–Infant Research on Environmental Chemicals (MIREC) Study Group and the MIREC Study staff for

their support and contribution to our study. We also thank the participants for their dedication to the study. The MIREC Study was funded by the Chemicals Management Plan of Health Canada, the Canadian Institutes of Health Research (MOP-81285), and the Ontario Ministry of the Environment. Y.O. and G.M.W. were funded by a grant from the Canadian Institutes of Health Research (MFE-140907 and MFE-339339-152889). J.M.B. and T.E. were funded by the U.S. National Institutes of Health/National Institute of Environmental Health Sciences (R01 ES024381).

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